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Various scenarios of the cell pattern formation in *Arabidopsis* lateral root

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Abstract

During lateral root (LR) development a coordinate sequence of cell divisions, accompanied by a change of the organ form takes place. Both the order of anatomical events and morphological features may vary for individual primordia. At early stages of LR primordia development oblique division walls are inserted in cells that are symmetrically located on both sides of the axis of the developing LR primordium, and thereby allow for the protrusion of the LR. We hypothesize that both oblique cell wall insertion and continuous changes in primordium form could be a consequence of a local change in stress distribution in the region of the LR initiation.

Keywords: lateral root formation; sequence of cell divisions; plant organ morphology

Introduction

Lateral roots (LRs) develop from the pericycle cells that locally acquire competence to form postembryonic meristems. Primordia are initiated in a left-right alternating pattern [1] and the process is auxin-regulated [2]. Eight stages of the LR formation in *Arabidopsis* have been identified [3], during which a sequence of cell divisions takes place. The initiation is morphologically detected when founder cells begin undergoing asymmetrical anticlinal divisions [4]. After the first periclinal divisions two cell layers are formed; an outer layer (OL) and an inner layer (IL). Further divisions lead to the formation of subsequent cell layers and to the increasing number of cells. A primordium expands by growing through the parent root tissues to emerge through the epidermis [3]. At this stage the geometry and cell patterning of the LR apex are fixed [5] and resemble those observed in the main root.

The proposed [3] scenario of events occurring during primordium development serves as a point of reference in studies on the LR formation. Based on these results we report and consider new morphological features that have hitherto not been described, with an emphasis on the occurrence of obliquely-oriented cell walls, irregularities in the cell division order, and primordia shapes. We show how a consideration of the reported features can provide new insights about LR formation and its possible relation to mechanical stress distribution.

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Material and methods

Arabidopsis wild type Col-0 (WT) and transgenic lines DR5::GFP, DR5::GUS, pPIN1::PIN1:GFP, AUX1::YFP were used to analyze LR development. Seedlings were grown as described in [6]. For microscopic observations samples were used fresh or cleared in chloral hydrate. The roots were observed using phase contrast and Nomarski microscopy. Images of 1740 LR primordia of the mentioned accessions (Tab. 1) were analyzed in their axial planes. Identification of developmental stages of LR formation followed that of [3].

Results and discussion

A sequence of primordia at subsequent stages is shown in Fig. 1a–l. At the site of the LR initiation two founder cells are visible, separated by the wall perpendicular to the root axis, although in some primordia this wall is oblique (Fig. 1a). In such cases the wall orientation is preserved at further stages (Fig. 1c, Fig. 2a). In most primordia a stage of development could be identified and sequence of divisions followed after Malamy and Benfey [3], yet, in some cases a departure from this order was found. Very seldom (2 of the 1740 analyzed, one in DR5::GFP line and one in the AUX1::YFP line), a periclinal division of a single pericycle cell occurred (Fig. 1b), instead of a series of anticlinal divisions, typically preceding formation of the two-layer primordium. In this case a single cell might have become a founder cell. This observation is in accordance with the suggestion [7] that the number of pericycle cells capable of becoming founder cells is 1 to 3.

Tab. 1 Quantitative comparison of morphological features observed in LR primordia in the analyzed lines.

	DR5::GFP	DR5::GUS	pPIN1::PIN1:GFP	AUX1::YFP	Col-0	All accessions
number of LRs	356	404	217	376	387	1740
relative number of LRs	20.5%	23.2%	12.5%	21.6%	22.2%	100.0%
changed division order	18 (5.1%)	29 (7.2%)	6 (2.8%)	12 (3.2%)	27 (7.0%)	number of cases (relative number of cases in the accession)
flattened apical part	38 (10.7%)	48 (11.9%)	14 (6.5%)	36 (9.6%)	40 (10.3%)	
asymmetrical shape	11 (3.1%)	21 (5.2%)	17 (7.8%)	36 (9.6%)	24 (6.2%)	

In later stages a change of division sequence was more frequent (5.29% of the 1740 analyzed cases) and it concerned mostly the insertion of anticlinal walls. In primordia at stages 2–4 anticlinal divisions preceding new layer formation occurred either in OL (Fig. 1d) or in IL (Fig. 1f,g,i). In some primordia a characteristic of stage 3 periclinal division took place in a cell of the OL neighboring an anticlinally divided cell of the IL (Fig. 1f). In other cases an anticlinally divided cell in the IL was found adjacent to a cell of the same layer that has undergone a regular periclinal division at stage 4 (Fig. 1i). Most of the changes were not exceptional and may be interpreted as a variation of the typical scenario. The early periclinal division of a single pericycle cell (Fig. 1b) is rather unique; more frequent are flip-flops at stages 2–4. The last may be associated with the overlapping of two subsequent stages and probably does not interfere with further development. Previous studies [8] showed that a program of cell divisions is not stereotypical and each individual primordium can pass through developmental stages with different numbers of cells.

From stage 2, walls perpendicular and parallel to the main root axis were inserted in the OL and IL (Fig. 1d–i). Orientation of these walls was adjusted to cell pattern of the main root. At stages 3 and 4 in cells located on the sides of the primordium axis, oblique walls were observed (Fig. 1g–i), which corresponded with a protrusion and emergence, and which led to formation of a dome-like shaped apex [6]. Derivatives of these cells are sharply pointed and situated close to the organ axis (Fig. 1l). In some cases a border between the cells derived from OL and IL is clearly visible (Fig. 1l) and a characteristic difference in the cell arrangement and cells' shapes occurs. The cells of OL are large and form layers aligned along the primordium outline, while the central cells of IL are elongated and form a column parallel to the organ axis. The cells of IL at the sides of the column are either arranged in accordance with the cells of OL or elongated and sharply tapered (Fig. 1l). Inclined cell walls have been reported in LR primordia of radish [9] in which oblique divisions at early stages led to formation of cuneiform cells, and in *Arabidopsis* [6], in which such walls were interpreted as a manifestation of a change of the principal directions of growth (PDG) [10] within the de novo formed meristem.

Under external mechanical stress plant cells divide in one of the principal directions of stress (PDS) [11]. As division walls are usually inserted along PDG [6], PDS and PDG may coincide [12]. Thus, the oblique divisions in a primordium may indicate a local reorientation of PDG resulting from redistribution of stress, as suggested by Lintilhac [13]. Such oblique walls are added no sooner than at stage 3 (Fig. 1g–i), which corresponds with the hypothesis [6] of a switch from a field of growth of the main root to the separate field of LR, and with the observation, that the isolated primordium is not capable of growth until it has developed 3 to 5 layers of cells [14].

On the sides of LR primordia strongly marked anticlinal cell walls are visible. First appearing in young primordia (Fig. 1e), they remain evident at later stages (Fig. 1h,i,k,l). Fates of cells in these side regions are similar either in observation [3] or in modeling [5,6]: they are formed at early stages and soon thereafter they stop dividing. The external walls of these cells seem to constitute LR boundaries separating the LR from the parent root. Such a separation may be related to the specific distribution of stress in the site of the LR formation. Most primordia show rather regular geometry; they are symmetrical in reference to their axes and they assume dome-like forms, yet, a number of deformed primordia also occur (see Tab. 1). In 10.11% of all analyzed primordia flattened apical portions with bent vascular bundles of the main root (Fig. 1j) were observed, whereas others manifested asymmetrical shapes (6.26% of the 1740 analyzed cases, Fig. 1k) or lack a regular shape at the tip (single cases, Fig. 1l). In LRs whose apical parts have emerged outside the parent root surface, such changes are hardly ever observed. This suggests that a changed geometry of primordia may be the result of mechanical stress from the surrounding tissues [15].

In few primordia at comparable stages, we observed significant differences in their shapes and dimensions. In Fig. 2a–c primordia consisting of two cell layers (stage 2) are shown. Two primordia (Fig. 2a,b) are rather flat, while in the third (Fig. 2c) a clear protrusion has appeared. Also, sizes and number of cells involved with LR formation changed from 4 cells (Fig. 2a) to about 14 cells (Fig. 2b,c). Moreover, the cells of the primordium in Fig. 2a are large in comparison to the two remaining cases. The variation in numbers of cells may be related to the varying number of the founder cells [7] and to the perturbed sequence of cell divisions.

In few cases (12 of the 1740 analyzed, single cases in particular accessions) atypical positioning of the LR primordia was observed. In Fig. 2d,e examples are shown in which two neighboring primordia are formed very close to each other. In some cases they appear on the same side

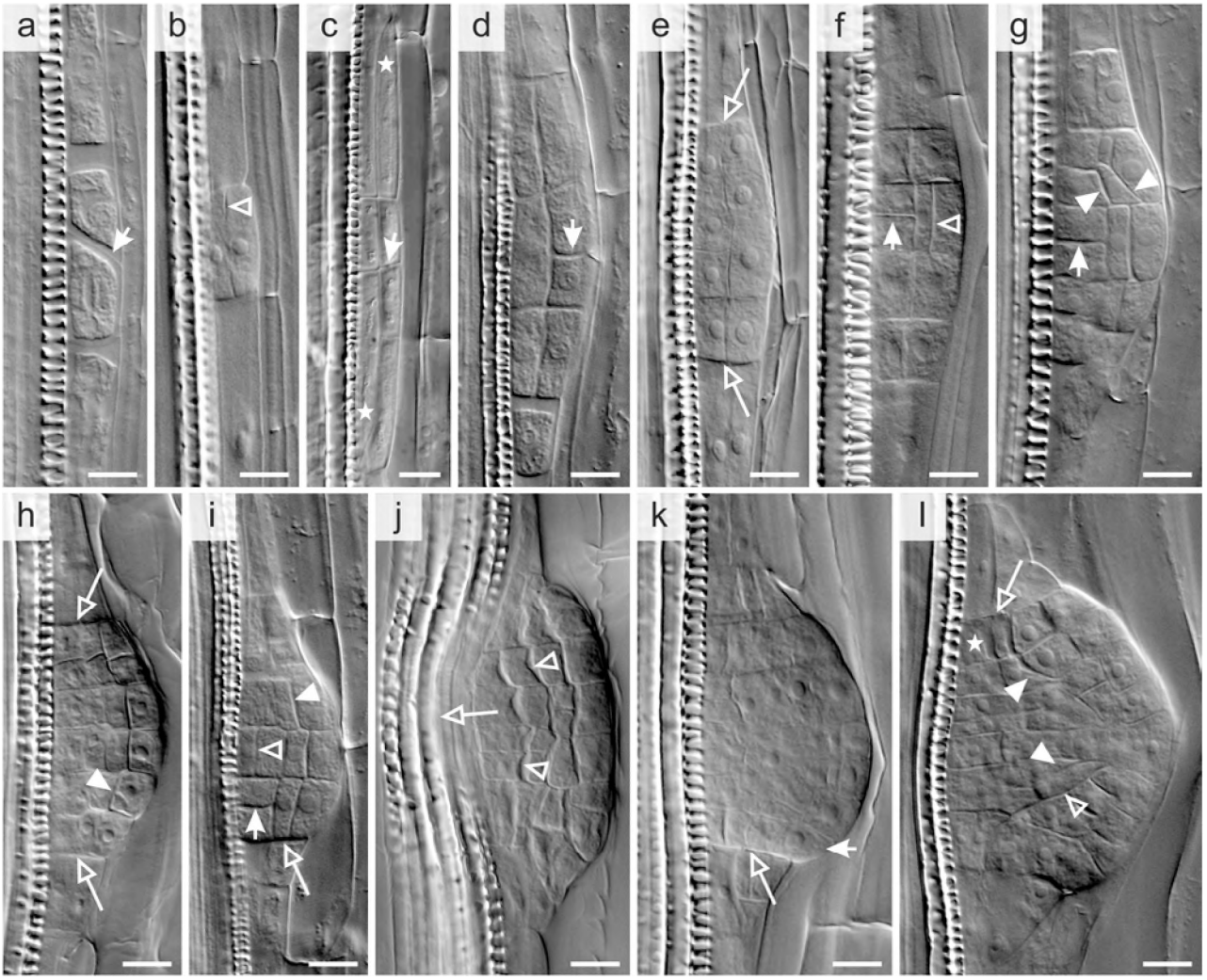

Fig. 1 LR primordia at consecutive stages: **a** – stage 1; **b** – stage 1/2; **c–e** – stage 2; **f,g** – stage 3; **h,i** – stage 4; **j–l** – presumably stages 5, 6, 7, respectively. **a** Oblique orientation of the central anticlinal wall (short arrow), preserved at stage 2 (**c**; short arrow). **c** Curved cell walls at the flanks (asterisks). A changed order of divisions: **b** Early periclinal division wall in pericycle (open arrowhead). Anticlinal division wall (short arrow) in OL (**d**) and in IL (**f,g,i**). **f** First periclinal division in OL (open arrowhead) characteristic of stage 3. **i** First periclinal division in IL (open arrowhead) characteristic of stage 4. **e,h,i,k,l** Strongly marked borders between the main root and primordium (arrows). **g–i** Oblique periclinal division walls (solid arrowheads) on the sides leading to sharply pointed cells (**l**; solid arrowheads) formation. **j** Local bending of vascular bands (arrow), curved periclinal walls (open arrowheads). A disturbed symmetry in the primordium shape: flattened apical part (**j**), stronger growth of the lower part (**k**; short arrow), conically shaped primordium with regular cell arrangement at the sides (**l**; asterisk), border between OL and IL (open arrowhead). Nomarski optics. Scale bars: 10 μm. **a,d,g,k** WT; **b,e,h,j** DR5::GFP; **c** AUX1::YFP; **f,i,l** DR5::GUS.

of the main root axis (Fig. 2d), while in other cases on the opposite sides (Fig. 2e). Typically, subsequent primordia are formed alternately on the left and right side [1] and they are distributed at more or less regular distances from each other, which is correlated with fluctuations in auxin distribution along the parent root [2]. However, LR density along the parent root was shown to be characteristic for individual accessions of *Arabidopsis* [1]. Root branching may be also controlled by mechanisms of lateral inhibition, caused by a competition between initiation and development for auxin [16]. As new LR initiation may be induced on the outside of the curve of the bent root [17], positioning may be also regulated by external mechanical signals.

In Tab. 1 a quantitative summary of the morphological features observed in LR primordia of all analyzed lines is presented. Slight differences in the relative number of cases between particular accessions may result from the fact that primordia were observed with the use of various microscopy techniques. For example, the roots of the pPIN1::PIN1:GFP line were mostly observed using phase contrast, which not allways allowed for clear identification of the cell pattern. That is why in this line there were only few primordia, in which a changed division order was observed in comparison with other lines. A sequence of events during the LR formation in *Arabidopsis* was described in [3]. Here, we have indicated and assessed additional features observed in the

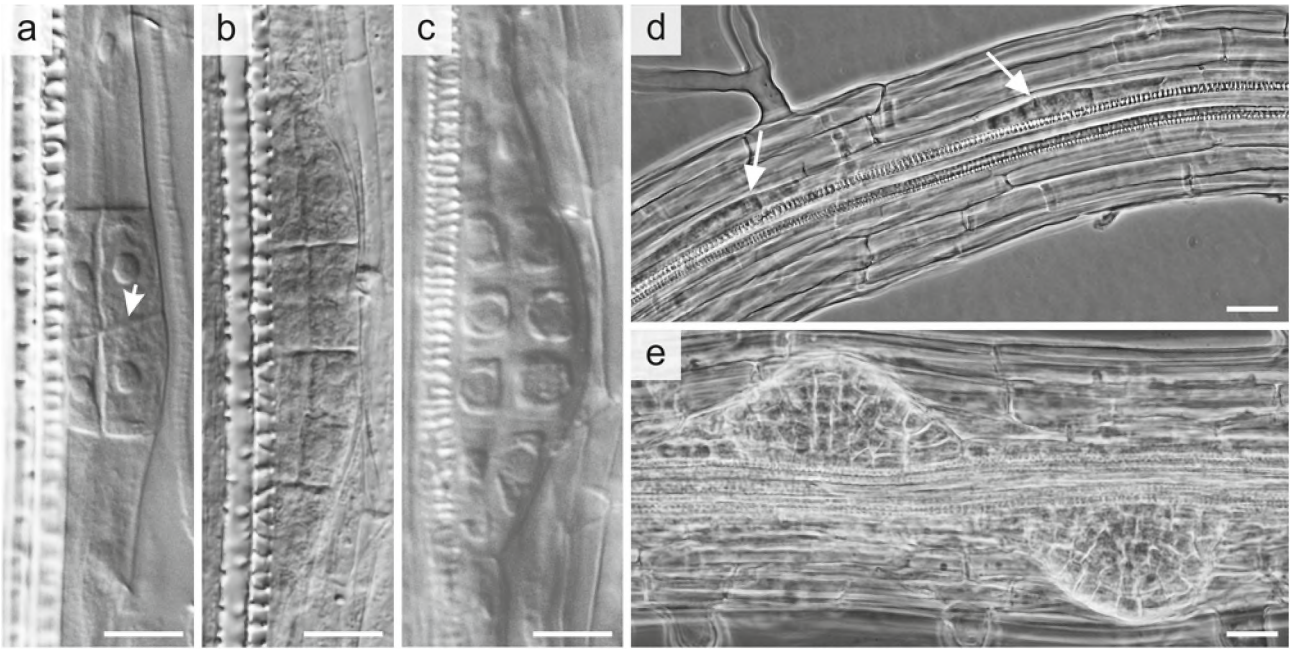

Fig. 2 Morphological features of the primordia. **a** Small primordium with few cells; oblique central wall in two layers (short arrow). **b** Large and flat primorium. **c** Small primordium forming a slight protrusion. Two closely formed LR primordia (arrows) on one (**d**) or opposite (**e**) sides of the root axis. **a–c** Nomarski optics; **d,e** phase contrast. Scale bars: **a–c** 10 μm; **d,e** 20 μm. **a,b,d** DR5::GFP; **c** DR5::GUS; **e** pPIN1::PIN1:GFP.

cell pattern of either WT or transgenic lines with comparable frequency. Some of these features are likely correlated with the stress distribution within the region of the LR initiation. In order to verify this hypothesis, further studies are required to investigate the effects of mechanical factors on morphology of *Arabidopsis* roots. The challenge for the future will also be to study the dynamic character of the mechanical stress distribution in the site of the LR formation.

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Authors' contribution

The following declarations about authors' contributions to the research have been made: designed research: JSP, IP; performed experiments: JB, JSP; wrote the manuscript: JSP, IP.

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